The GnRH analogue dephereline given in a fixed-time AI protocol improves ovulation and embryo survival in dairy cows

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Abstract

This study compares the fertility effects of inducing ovulation using the GnRH analogue, dephereline, versus natural GnRH at the end of a 5-day progesterone(P4)-based protocol for fixed-time artificial insemination (FTAI) in heat-stressed and non-heat stressed lactating dairy cows. Cows were given GnRH (GnRH group, n = 369) or dephereline (DEPH group, n = 379) and were inseminated 14-20 hours later. Dephereline treatment increased corpus luteum (CL) size on Day 7 post-AI compared with GnRH ($P < 0.0001$) while a one-mm increase in CL size was found to give rise to a 1.1-fold increase in the pregnancy rate at FTAI ($P = 0.001$). Based on odds ratios, the interaction between treatment and heat stress had a significant effect on the ovulation failure rate ($P < 0.01$). This meant that relative to non-heat-stressed GnRH-treated cows, ovulation failure was 2.9 times more likely in heat-stressed GnRH-treated cows ($P = 0.001$), 0.3 times less likely in non-heat-stressed DEPH-treated cows ($P = 0.04$) and was similar in heat-stressed DEPH-treated cows. Further, non-heat-stressed DEPH-treated cows were more likely to conceive by a factor of 1.6 than the remaining cows ($P = 0.03$). Finally, GnRH-treated multiparous cows were 9.9 times more likely to suffer pregnancy loss than the remaining cows ($P = 0.03$). Our results indicate that, compared to treatment with GnRH, dephereline reduced the risk of ovulation failure and consequently increased the pregnancy rate under heat stress conditions. In multiparous cows, dephereline treatment also reduced the negative age effect on pregnancy maintenance.

Keywords: bovine; ovulation; conception rate; early fetal loss

1. Introduction

Low fertility and pregnancy loss during the late embryonic/early fetal period are major constraints in high producing dairy cows (Diskin et al., 2011; López-Gatius, 2012). After AI, reduced progesterone production by the corpus luteum (CL) may compromise both embryo implantation and growth (López-Gatius and Garcia-Ispierto, 2010; Practice Committee of the American Society for Reproductive Medicine, 2012). Conversely, a normal functioning CL has been related to an increased pregnancy rate and a reduced risk of early pregnancy loss (Ricci et al., 2017; Garcia-Ispierto et al., 2018). Luteal insufficiency is induced by factors such as metabolic stress (e.g., clearance of steroid hormones related to high milk production (Wiltbank et al., 2006)) and heat stress (De Rensis et al., 2015). The incidence of anestrus, or lack of cyclicity, is also dramatically increased by a warm environment (López-Gatius, 2003; De Rensis et al., 2017). A defining pattern in anestrous cows (Peter et al., 2009; Santos et al., 2016) is a lack of ovarian progesterone production. This determines that progesterone(P4)-based protocols for inducing ovulation for fixed-time AI (FTAI) seem to better resolve anestrus related to a warm environment than gonadotropin releasing hormone(GnRH)-based protocols (Macmillan, 2010; Peter et al., 2009; De Rensis et al., 2015). However, P4-based protocols have not fully resolved the problem of ovulation failure in anestrous cows (Garcia-Ispierto and López-Gatius, 2014; Garcia-Ispierto et al., 2018). Indeed, the likelihood of ovulation failure may be up to 4 times higher in cows under heat stress showing clear estrous signs including the presence of an ovulatory follicle (López-Gatius et al., 2005; López-Gatius and Hunter, 2017). Further, it has been well documented that during the warm period as compared to the cool period of the year, there is a greater incidence of implantation failure and late embryonic/early fetal mortality (Garcia-Ispierto et al., 2006; Grimard et al., 2006; Alhussein et al., 2018).

The pre-ovulatory follicle synthesizes and secretes increasing quantities of estradiol during the last three or four days of an estrous cycle. In due course, a threshold concentration of estradiol causes both the behavioral response of estrus and a surge in GnRH release from the hypothalamus. This results in a peak of gonadotropins, especially of LH, which triggers ovulation 20-30 h later (Hunter, 2003). An aqueous solution of the natural deca-peptide GnRH applied intra-muscularly causes LH and FSH release within 30 min of treatment. This is why GnRH is an integral part of synchronizing regimes of ovulation in FTAI programs (Thatchwer et al., 1993; Peters, 2005; Carvalho et al., 2018). Dephereline (gonadorelin [6-D-Phe]) is a synthetic analogue of GnRH in which glycine is replaced by D-phenylalanine at position 6 in the amino acid sequence. Dephereline shows a stronger and longer period of action than natural GnRH (Busch, 1986). To our knowledge, so far only one study has assessed the use of dephereline in cattle. In this study conducted in 1846 cows in spontaneous estrus, treatment with the synthetic hormone increased ovulation and pregnancy rates in 11 and 8, respectively, of the 12 herds included in the study (Böhme et al., 1988). However, the use of dephereline to induce ovulation within an FTAI protocol has not yet been explored. The aim of the present study was to compare the fertility effects of dephereline versus natural GnRH given at the end of a 5-day P4-based FTAI protocol in in heat-stressed and non-heat stressed lactating dairy cows. The impact of dephereline on pregnancy loss was also examined.

2. Material and Methods

2.1. Cattle and herd management

This study was performed on a commercial Holstein-Friesian dairy herd in northeastern Spain. During the study period (May 2017 to June 2018), the mean number of lactating cows in the herd was 220 and mean annual milk production was 10,150 kg per cow. The mean annual culling rate was 30%. Cows were grouped according to age (younger, primiparous plus secundiparous versus older, more than two lactations), milked two times daily, and fed complete rations. All cows were artificially inseminated and the herd was subjected to a weekly reproductive health program, as described elsewhere (Garcia-Ispierto and López-Gatius, 2014; López-Gatius and Hunter, 2017). Only healthy cows free of detectable reproductive disorders and free of clinical diseases during the study period (Days −7 to 56 of insemination) were included. Exclusion criteria were the following disorders: mastitis, lameness, digestive disorders and pathological abnormalities of the reproductive tract detectable by ultrasonography.

2.2. Experimental design

All procedures were approved by the Ethics Committee on Animal Experimentation of the University of Lleida (license numbers CEEA.06-01/12 and CEEA.09-01/13).

During the weekly reproductive program visit, open cows more than 60 days in milk were alternately assigned on a weekly rotational basis to the groups: GnRH (n = 369) or DEPH (n = 379). All cows were treated with a controlled internal drug release (CIDR, insert; Zoetis, New York, NY, USA) plus GnRH (100 μg gonadorelin diacetate tetrahydrate i.m.; Cystoreline, CEVA Salud Animal, Barcelona, Spain) upon CIDR insertion. The CIDR was left in place for 5 d, and the animals were also given cloprostenol (500 μg i.m.; PGF Veyx Forte, Ecuphar, Barcelona, Spain) on CIDR removal. Twenty-four hours later the cows received a second cloprostenol dose. Thirty six hours later, cows in the GnRH group were given a second GnRH dose while cows in the DEPH group were given dephereline (100 μg gonadorelin acetate [6-D-Phe] i.m; Gonavet Veyx, Ecuphar, Barcelona, Spain). Cows in both groups were inseminated 50-56 hours after CIDR removal.

All cows in the herd were FTAI by the same technician with frozen-thawed semen from 10 bulls 14-20 hours after the second GnRH or dephereline dose. Pregnancy diagnosis was performed by ultrasound 28 d post-AI. Pregnancy was confirmed 56 d post-AI in pregnant cows. Pregnancy loss was recorded when the second pregnancy diagnosis proved negative. Cows diagnosed as not pregnant underwent a second round of treatment, but always within the same group, GnRH or DEPH. Thus, the study sample included 748 inseminations performed in 302 cows. All gynecological exams and pregnancy diagnoses were performed by the last author by transrectal ultrasonography using a portable B-mode ultrasound scanner equipped with a 5-10 MHz transducer (E.I. Medical IBEX LITE; E.I. Medical Imaging, Loveland CO, USA). Each ovary was scanned in several planes by moving the transducer along its surface to identify luteal structures, and the number and location of corpora lutea were recorded. Scanning was then run along the dorso/lateral surface of each uterine horn. The presence of twins was recorded when two embryos were observed in different positions within one uterine horn on two scan screens, two embryos were simultaneously present on the screen (unilateral twin pregnancy), or when one embryo could be seen in each uterine horn (bilateral twin pregnancy).

2.3. Data collection and statistical analysis

Ovarian follicular structures larger than 10 mm in diameter and the absence or presence of one or more CL at least 10 mm in diameter were assessed by ultrasonography immediately before AI, and 7 days after AI to confirm ovulation. A cow was classified as ready for service at AI when the CL was either less than 10 mm or non-detectable, the diameter of the largest follicle was greater than 10 mm and the uterus was highly turgid and contractile to the touch (López-Gatius, 2012). Corpus luteum size was recorded in ovulating cows 7 days post-AI taken as the mean of two measurements approximating the greatest length and width. In the case of cavity CL, the mean value of the luteal wall was also recorded. In the case of two corpora lutea, the maximum measurements of the greatest CL were used for the analyses. Pregnancy rate was defined as the percentage of cows that became pregnant at FTAI out of the total number of cows in the corresponding group. The maximum temperature–humidity index (THI) on the day of treatment (60 h after CIDR removal) was used to evaluate the effects of heat stress (THI values higher than 72 (De Rensis et al., 2015)) on subsequent reproductive performance. It should be noted that in our geographical region, a clear negative effect of heat stress on the reproductive performance of lactating dairy cows has been extensively described (López-Gatius, 2003; Garcia-Ispierto et al., 2006; López-Gatius and Hunter, 2017).

The following data were recorded for each animal: parturition and treatment dates; parity (primiparous versus multiparous); treatment (GnRH or DEPH); maximum THI at treatment (≤ 72 versus > 72); milk production at AI (low producers < 40 kg versus high producers ≥ 40 kg); days in milk at AI (DIM; < 90 postpartum versus ≥ 90 d); CL at the start of the synchronization protocol; ovulation failure (absence of a CL 7 days after AI); multiple ovulation (presence of two or more CL 7 days after AI); sire; pregnancy after FTAI; presence of twins after FTAI; and pregnancy loss. Since CL size was not significantly different between the GnRH- (18.7 ± 5.4 mm) and DEPH (20 ± 4.8 mm) treated cows, only data for CL without a cavity in single ovulating cows and the larger CL in double ovulating cows were included in the analyses.

Overall reproductive performance for the two treatment groups was evaluated using the chi-squared test. The effects of treatment on the size of CL without cavities were analyzed by the Student's t-test. The effects of treatment group on ovulation failure, double ovulation, pregnancy, twin pregnancy and pregnancy loss rate were analyzed by logistic regression (logistic procedure of PASW Statistics for Windows Version 18.0, SPSS Inc., Chicago, IL, USA) adjusting for lactation, days in milk, milk production, heat stress, sire and technician. The estimates and Wald 95% limits were used to calculate odds ratios and 95% confidence intervals (CI). Explanatory variables and interactions were assessed using the backward elimination procedure; variables that significantly affected ovulation or pregnancy rate remaining in the model. Significance was set at $P < 0.05$. Values are expressed as the mean ± standard deviation (SD). The factors entered in the model as independent dichotomous variables (where 1 denotes presence and 0 denotes absence) were parity (multiparous), CL at the start of the synchronization protocol and heat stress at treatment (THI > 72). Treatment, days in milk at AI, milk production at AI and sire (class variables) were considered factors in the analyses. Possible interactions between treatment and the dichotomous variables heat stress, CL at the start of the synchronization protocol and parity were also analyzed. For the dependent variable double ovulation, only ovulating cows were included in the analysis. For the dependent variables twin pregnancy and pregnancy loss, only pregnant cows were included in the analysis.

Regression analyses were conducted according to the method of Hosmer and Lemeshow (Hosmer and Lemeshow, 1989) using the logistic procedure of PASW Statistics for Windows Version 18.0 (SPSS Inc., Chicago, IL, USA). Basically, this method consists of five steps as follows: preliminary screening of all variables for univariate associations; construction of a full model using all the significant variables arising from the univariate analysis; stepwise removal of non-significant variables from the full model and comparison of the reduced model with the previous model for model fit and confounding; evaluation of plausible interactions among variables; and assessment of model fit using Hosmer-Lemeshow statistics. Variables with univariate associations showing P values < 0.25 were included in the initial model. Model reduction continued until only significant terms according to the Wald statistic remained in the model at P < 0.05.

3. Results

Mean milk production at AI, days in milk at AI, number of lactations and number of inseminations were 36.0± 8.3 kg, 166.0 ± 100.7 days, 2.6 ± 1.5 lactations and 4.0 ± 3.0 inseminations, respectively (mean ± SD). All cows were considered to be ready for insemination at FTAI. No cavity CL was recorded in double ovulating cows. Two hundred and sixteen (28.9%) of the 748 cows enrolled became pregnant following FTAI. No cows suffering ovulation failure became pregnant. Twin pregnancy was recorded in 8.8% of the cows (19/216) and 18.5% of the cows (40/216) undergoing pregnancy loss. Values of each independent variable for each treatment and effects of the different treatments on each dependent variable are shown in Table 1.

According to chi-squared tests, ovulation failure and pregnancy loss rates were significantly lower and the pregnancy rate significantly higher in the DEPH group (P < 0.05). Corpora lutea without cavities on Day 7 post-AI were observed in 492 cows (253 DEPH; 239 GnRH). CL size was significantly reduced in response to the GnRH treatment (19.2 ± 4.4) mm compared with the DEFH treatment (21.9 ± 4.3 mm) (Student's t-test: $P < 0.0001$).

Based on odds ratios (Table 2), the interaction between treatment and heat stress had a significant effect on the ovulation failure rate ($P < 0.01$). This meant that compared to the rates recorded in non-heat-stressed GnRH-treated cows, ovulation failure was more likely in heat-stressed GnRH-treated cows by a factor of 2.9 ($P = 0.001$), was less likely in non-heat-stressed DEPH-treated cows by a factor of 0.3 ($P = 0.04$) and was similar in heat-stressed DEPH-treated cows. Ovulation failure was 0.51 times less likely in multiparous cows ($P = 0.02$; Table 2). The interaction between treatment and heat stress had a significant effect on the pregnancy rate ($P = 0.01$) (Table 3). The treatment-heat stress interaction determined that non-heat-stressed DEPH-treated cows were 1.6 times more likely to conceive than the remaining cows ($P = 0.03$). The interaction between treatment and parity had a significant effect on the pregnancy loss rate ($P = 0.01$) (Table 4). The treatment-parity interaction determined that GnRH-treated multiparous cows were more likely to suffer pregnancy loss by a factor of 9.9 than the remaining cows ($P = 0.03$).

No significant effects of treatment on the double ovulation or twin pregnancy rate were identified by binary logistic regression. Since treatment affected CL size in cows with a CL without cavity, a further binary logistic analysis was performed with pregnancy rate at FTAI as the dependent variable for cows with a CL without a cavity (n = 482).

In this analysis, a one-mm increase in CL size was found to give rise to a 1.1-fold increase in the pregnancy rate at FTAI (odds ratio: 1.1; 95% confidence interval: 1.02-1.09; P = 0.001).

4. Discussion

As far as we know, no prior study has revealed the efficacy of dephereline used as an ovulation inducer at the end of an FTAI protocol. In our study, this GnRH analogue improved ovulation and pregnancy rates under heat stress conditions compared to the use of natural GnRH. Moreover, pregnancy losses in multiparous cows following dephereline treatment were reduced to similar figures to those recorded in primiparous cows.

More than 40% of inseminations (312/748) were performed under heat stress conditions (THI > 72) indicating that in temperate regions such as Spain, heat stress may be incurred over a period longer than just the summer. Heat stress dramatically disrupts the reproductive performance of cows, increasing the incidence of anestrus and therefore the number of days open, reducing the conception rate (De Rensis et al., 2015, 2016; Hansen and Arechiga, 1999; Kadzere et al., 2002). There is thus an urgent need for hormone treatments able to offset the negative effects of heat stress in high producing dairy herds in warm regions. Our findings indicate that dephereline may be beneficial within a short P4-based FTAI protocol. In heat-stressed cows, treatment reduced the incidence of ovulation failure and consequently increased the pregnancy rate when compared to GnRH. Although plasma P4 concentrations were not determined, our analysis of data for only CL without a cavity proved valuable and we were able to link a larger CL size in ovulating cows treated with dephereline to a higher pregnancy rate at FTAI. Probably, in our DEPH group the risk of sub-luteal function related to heat stress was reduced (De Rensis et al., 2015).

In well-managed dairy herds, non-infectious causes of pregnancy termination before the fetus is viable are more common than infectious causes (Grimard et al., 2006; López-Gatius, 2012). Age, or lactation number, is a leading cause of pregnancy loss during the early fetal period (Labèrnia et al., 1996; López-Gatius et al., 2004). The long lasting effects of dephereline observed here could be the outcome of enhanced early CL function, thus inducing the ovary to produce more P4. Since the beneficial effects of treatment were produced throughout the year in our older cows, it was improved CL function that likely overcame the negative effects of P4 clearance related to high milk production in multiparous cows (Wiltbank et al., 2006). If dephereline has a stronger longer-lasting action than natural GnRH (Busch, 1986), it would be interesting to examine the effects of increasing dephereline doses, either at AI or during the early luteal phase, on subsequent fertility and pregnancy maintenance. In essence, the efficiency of natural GnRH seems to be limited to the standard 100 µg dose. Some authors have observed that while increased GnRH doses enhance pituitary LH release, a double dose given at induced estrus leads to a subsequent lower plasma P4 concentration than in control cows receiving a single dose (Colazo et al., 2009). Similarly, as GnRH treatment at pregnancy diagnosis in cows carrying twins favors pregnancy maintenance (Bech-Sàbat et al., 2010; López-Gatius et al., 2017), the effects of a double GnRH dose on pregnancy survival in twin pregnancies was recently investigated (Garcia-Ispierto and López-Gatius, 2018). However, in high producing dairy cows carrying twins, a single or double GnRH dose given at pregnancy diagnosis returned similar pregnancy loss rates and reduced the risk of pregnancy loss up to three times compared to untreated cows (Garcia-Ispierto and López-Gatius, 2018).

Some synchronization protocols for FTAI may increase the risk of twin pregnancy (Andreu-Vázquez et al., 2012a). Carrying twins is a main factor jeopardizing pregnancy maintenance and reducing the lifespan of the dairy cow (Andreu-Vázquez et al., 2012b). In our study, dephereline treatment neither increased the risk of double ovulation nor the twin pregnancy rate.

As an overall conclusion, dephereline emerged here as a good alternative to GnRH to induce ovulation. Treatment with this GnRH analogue reduced the risk of ovulation failure and consequently increased the pregnancy rate under heat stress conditions. In addition, we also noted that treatment in multiparous cows diminished the detrimental effects of age on pregnancy maintenance. More work is needed to assess the dose dependency of the beneficial effects observed.

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Tables

Table 1. Independent variables recorded at AI for each treatment and effects of the treatments on each dependent variable (n = 748).

Treatment	GnRH (n = 369)	DEPH (n = 379)
Independent variables		
Parity (multiparous)	270 (73.2%)	290 (76.5%)
Milk production (> 40 kg)	111 (30.1%)	105 (27.7%)
Days in milk (>90 d)	271 (73.4%)	268 (70.7%)
Heat stress (max THI > 72)	144 (39.0%)	168 (44.3%)
CL at the start of the synchronization protocol	244 (66.1%)	276 (72.8%)
Dependent variable*		
Ovulation failure	43 (11.7%)[a]	15 (4.0%)[b]
Double ovulation**	97 (29.8%)	111 (30.5%)
Pregnancy rate at FTAI	89 (24.1%)[a]	127 (33.5%)[b]
Twin pregnancy rate***	7 (7.9%)	12 (9.5%)
Pregnancy loss rate***	23 (25.8%)[a]	17 (13.4%)[b]

*Values with different superscripts within rows differ according to Chi-squared tests ($P < 0.05$).

**Percentages in ovulating cows.

***Percentages in pregnant cows.

Treatments (all cows were FTAI 50-56 h after CIDR removal):

GnRH: cows given GnRH 36 h after CIDR removal.

DEPH: cows given dephereline 36 h after CIDR removal.

Table 2. Odds ratios of the ovulation failure variables included in the final logistic regression model (n = 748).

Factor	Class	n	% ovulation failure	Odds ratio	95% confidence interval	P
Parity	Primiparous	23/188	12.2	Reference		
	Multiparous	35/560	6.3	0.51	0.29-0.91	0.02
Treatment x THI* interaction	0 (≤ 72)	16/225	7.1	Reference		< 0.01
	0 (> 72)	27/144	18.8	2.9	1.5-5.7	0.001
	1 (≤ 72)	5/211	2.4	0.3	0.1-0.9	0.04
	1 (> 72)	10/168	6.0	0.8	0.3-1.8	0.3

Hosmer and Lemeshow Goodness-of-fit test = 22.3; 2 df, P = 0.95.

R^2 Nagelkerke = 0.14.

*Treatment: 0, GnRH; 1, dephereline. THI: maximum temperature-humidity index at treatment (between parentheses).

Treatments (all cows were FTAI 50-56 h after CIDR removal):

GnRH: cows given GnRH 36 h after CIDR removal.

DEPH: cows given dephereline 36 h after CIDR removal.

Table 3. Odds ratios of the conception rate variables included in the final logistic regression model (n = 748).

Factor	Class	n	% conception rate	Odds ratio	95% confidence interval	P
Treatment x THI[*] interaction	0 (≤ 72)	62/225	27.6	Reference		0.01
	0 (> 72)	27/144	18.8	0.8	0.4-1.4	0.4
	1 (≤ 72)	87/211	41.2	1.6	1.03-2.5	0.03
	1 (> 72)	40/168	23.8	0.8	0.5-1.4	0.5

Hosmer and Lemeshow Goodness-of-fit test = 21.3; 2 df, P = 0.94.

R^2 Nagelkerke = 0.15.

[*]Treatment: 0, GnRH; 1, dephereline. THI: maximum temperature-humidity index at treatment (between parentheses).

Treatments (all cows were FTAI 50-56 h after CIDR removal):

GnRH: cows given GnRH 36 h after CIDR removal.

DEPH: cows given dephereline 36 h after CIDR removal.

Table 4. Odds ratios of the pregnancy loss variables included in the final logistic regression model (n = 216).

Factor	Class	n	% pregnancy loss	Odds ratio	95% confidence interval	P
Treatment x parity	0 primiparous	1/22	4.5	Reference		0.01
	0 multiparous	22/67	32.8	9.9	1.2-79.0	0.03
	1 primiparous	6/35	17.1	4.5	0.5-41.0	0.17
	1 multiparous	11/92	12.0	2.6	0.32-21.7	0.36

Hosmer and Lemeshow Goodness-of-fit test = 21.2; 2 df, P = 0.93.

R^2 Nagelkerke = 0.15.

*Treatment: 0, GnRH; 1, dephereline.

Treatments (all cows were FTAI 50-56 h after CIDR removal):

GnRH: cows given GnRH 36 h after CIDR removal.

DEPH: cows given dephereline 36 h after CIDR removal.